a range of morphologicial effects indicating a significant modification of the functioning of the actin cyctoskeleton, with effects on both cell division and size / shape. This is the first time a clear effect has been observed by the expression of a heterologous tropomyosin in S. cerevisiae and indicates the short N. crassa tropomyosin may have unusual actin-regulatory properties as seen previously for an similar-sized artificial construct based on the yeast TPM1.

1903-Pos Board B673
Structural Analysis of Smooth Muscle Tropomyosin Alpha and Beta Isoforms
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A large number of tropomyosin (Tm) isoforms function as gatekeepers of the actin filament, controlling the spatiotemporal access of actin-binding proteins to specialized actin networks. Residues ~40-80 vary significantly among Tm isoforms, but the impact of sequence variation on Tm structure and interactions with actin is poorly understood, since structural studies have focused on skeletal muscle Tmα. We describe structures of N-terminal fragments of smooth muscle Tm α and β (sm-Tmα and sm-Tmβ). The 2.0-Å structure of sm-Tmα81 (81-aa) resembles that of skeletal Tmα, displaying a similar super-helical twist matching the contours of the actin filament. The 1.8-Å structure of sm-Tmα98 (98-aa) unexpectedly reveals an anti-parallel coiled-coil, with the two chains staggered by only 4-aa and displaying hydrophobic core interactions similar to those of the parallel dimer. In contrast, the 2.5-Å structure of sm-Tmβ98, containing Gly-Ala-Ser at the N-terminus to mimic acetylation, reveals a parallel coiled-coil. None of the structures contains coiled-coil stabilizing elements, favoring the formation of head-to-tail overlap complexes in four out of five crystallographically independent parallel dimers. These complexes show similarly arranged 4-helix bundles stabilized by hydrophobic interactions, but the extent of the overlap varies between sm-Tmβ98 and sm-Tmα81 from 2 to 3 helical turns. The formation of overlap complexes thus appears to be an intrinsic property of the Tm coiled-coil, with the specific nature of hydrophobic contacts determining the extent of the overlap. Overall, the results suggest that sequence variation among Tm isoforms has a limited effect on actin binding, but could determine its gatekeeper function.

1904-Pos Board B674
Regulation of F-Actin Bundles by Triobp-4
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Parallel F-actin bundles, cross-linked by actin-bundling proteins, are key components of the cytoskeletal structure in cells, having a profound influence on many cellular processes. We recently reported that TRIOBP is a novel actin bundling protein. Mutations of human TRIOBP, a gene encoding multiple isoforms, are associated with human nonsyndromic deafness DFNB28. We found that *in vitro*, TRIOBP isoform 4 packs actin filaments into dense F-actin bundles. Mice with deletion of isoforms 4 and 5 of TRIOBP (TRIOBP-4/-5) exhibited profound deafness. The stereocilia of the inner ear hair cells in these mice failed to form rootlets and are more easily deflected and damaged. How TRIOBP forms uniquely dense actin bundles to affect the structure of inner ear hair cells remains enigmatic. Here, we show evidence that TRIOBP-4 forms rigid actin bundles by interacting with actin filaments from both lateral and longitudinal directions. We purified three fragments of TRIOBP-4 protein deleted for motifs 1 (R1) or 2 (R2), or both. Using high- and low-speed actin cosedimentation assays, we found that actin binding/bundling activities of R1 and R2 are regulated by different mechanisms. Furthermore, R1-deleted TRIOBP-4 fragment forms thin, sharp actin bundles, whereas R2-deleted TRIOBP-4 forms long, thick bundles, as examined by both total internal reflection (TIRF) microscopy and transmission electron microscopy (TEM). When overexpressed in cells, R2-deleted fragment shows co-localiztion with actin filaments, similar to intact TRIOBP-4. In contrast, R1-deleted TRIOBP-4 is uniformly expressed, showing no co-lcalization with F-actin. These results indicate that R1 and R2 motifs play distinct roles in actin-bundle formation. The structural basis for this regulation and the biological functions underlying this regulation are under further investigation.

1905-Pos Board B675
Myosin Isoform-Specificity of Contractile Elements Formed in Reconstituted Actomyosin Bundles
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Forces generated by the contraction of actomyosin bundles underlie diverse physiological processes including muscle contraction, cell migration and cytokinesis. While actomyosin force transmission is well understood in the context of organized sarcomeres found in striated muscle, very little is known about the nature of actomyosin force transmission in non-sarcomeric bundles such as those found in smooth and non-muscle cells. We recently showed that contractile elements spontaneously arise during the contraction of disordered actomyosin bundles reconstituted solely from actin filaments and smooth muscle myosin. In turn, the contraction of disordered actomyosin bundles arises from a several contractile elements linked in series resulting in a bundle contraction velocity linearly proportional to bundle length and a length-independent contractile force. The physical properties of the contractile elements, including the rate of contraction, maximum contractile strain and the maximum force generated, depended on the relative quantities of actin and myosin. Here, we demonstrate that reconstituted actomyosin bundles formed with skeletal muscle myosin form qualitatively similar contractile elements with similar dependencies on myosin concentration. However, the physical properties of the contractile units formed with skeletal muscle myosin dramatically differ from those formed with smooth muscle myosin, in a fashion consistent with differences in the myosin mechanochemistry and thick filament geometry of smooth and skeletal muscle myosin. Contractile elements formed by skeletal muscle myosin contract significantly faster, generate more force and can shorten to greater extents than those formed with smooth muscle myosin. Our results demonstrate that both smooth and skeletal muscle myosin isoforms can facilitate the spontaneous assembly of contractile elements within disordered actomyosin bundles and provide insight into the mechanisms underlying contraction in non-sarcomeric actomyosin bundles.

1906-Pos Board B676
Modeling and Simulation of Myosin-Dependent Rearrangement and Force Generation in an Actomyosin Network
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Non-muscle myosin II and actin constitute the major force-generating machinery of actomyosin networks, whose contractility is essential for processes that require cellular reshaping and movement such as cell migration and cell division. In particular, mechanical behaviors of actomyosin networks such as spontaneous rearrangements of networks into bundles are recognized as being fundamental to biological functions but the mechanochemical basis of the emergence of these functions is still unclear. Thus, to clarify the mechanochemical foundation of the emergence of cellular functions, understanding the relationship between actomyosin contractility and rearrangement of actomyosin networks is crucial. For this purpose, we present a new particulate-based model for simulating the motions of actin, non-muscle myosin II, and alpha-actinin. To confirm the model's validity, we successfully simulated sliding and bending motions of actomyosin filaments, which are observed as fundamental behaviors in dynamic rearrangement of actomyosin networks in migrating keratocytes. Next, we simulated the dynamic rearrangement of actomyosin networks. Our simulation results indicate that an increase in the density fraction of myosin induces a higher-order structural transition of actomyosin filaments from networks to bundles, in addition to increasing the force generated by actomyosin filaments in the network. We compare our simulation results with experimental results and confirm that actomyosin bundles bridging focal adhesions and the characteristics of myosin-dependent rearrangement of actomyosin networks agree qualitatively with those observed experimentally.

1907-Pos Board B677
A Biochemical Model for Sub-Cellular Waves of Actin Activity
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Sub-cellular waves / pulses of activity in the interior of cells have been observed in for example: Dictyostelium (Gerisch et al. Biophys J. Vol. 87 2004) and Neutorphils (Weiner et al. PLoS Biol. Vol. 5 2007). Models of such travelling waves are ubiquitous in ecology and neuroscience and involve the use of a wave generating component and a refractory component. We present a model developed within this structure that incorporates a wave generating

module based upon previous work on chemotactic regulatory kinetics and assumes actin growth serves as the necessary refractory negative feedback. We parametrically analyze the pattern forming capacity of this model through the use of a 'Local Perturbation Analysis' and simulations, and discuss its properties.

1908-Pos Board B678

A Two-Dimensional Model of Leading Edge Protrusion and Retraction Driven by Excitable Actin Dynamics

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Periodic patterns of protrusion and retraction have been observed along the leading edge of various cell types during spreading and motility, with many cells exhibiting wave-like propagation of protrusions along the cell membranes. Research has surged in this area as the reproducibility of these patterns offers opportunities to better quantify the regulatory mechanisms of actin polymerization at the leading edge. Previous works suggest possible roles for membrane curvature sensing proteins, actin filament nucleators, Rho signaling, myosin contraction, and severing in this process. To investigate cycles of protrusion and retraction we imaged the lamellipodia of XTC cells using Lifeact as a marker for actin, and analyzed the position of the leading edge using active contours. Constant retrograde flow indicated that protrusions and retractions were driven by fluctuations of the actin polymerization rate. We developed a 1D model of these actin dynamics as an excitable system in which a diffusive, autocatalytic activator causes free barbed end formation and actin polymerization; F-actin accumulation in turn inhibits further activator accumulation. According to the model, the leading edge protrusion rate spikes before the maximum of the free barbed end concentration. To gain mechanistic insight into how polymerization is converted into protrusion, we extended the model in 2D. F-actin dependent membrane motion and retrograde flow are both accounted for. We compare models of free barbed end generation to experiment using spatiotemporal correlations of leading edge velocity with concentrations of Arp2/3 complex, capping protein and VASP, as a function of distance from the leading edge. Our findings suggest that the Arp2/3 complex participates in an activation mechanism that includes additional diffusive components.

1909-Pos Board B679

Investigation of Molecular Level Stress-Strain Relationships in Systems of Entangled F-Actin by Combined Force-Measuring Optical Tweezers and Fluorescence Microscopy

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Actin plays a major role in cell structure, cell motility, vesicle and organelle transport, and muscle contraction. Actin's ability to play these major roles is a direct consequence of the intricate relationship between stress and strain in a variety of filamentous actin (F-actin) networks. A thorough understanding of the unique stress-strain relationships in complex F-actin networks at the *molecular-level* is currently lacking despite the importance of such networks to fields such as biomimetic material engineering and cell biology. Here we develop novel single-molecule instrumentation that combines dual-trap force-measuring optical tweezers with fluorescence microscopy to enable simultaneous characterization of intermolecular forces and molecular dynamics within F-actin networks. This instrumentation is combined with a novel technique in which single F-actin ''probes'' are used to apply molecular-level strains and measure induced stress within entangled F-actin systems while the deformations and dynamics of surrounding fluorescent-labeled filaments are simultaneously imaged. Specifically, the dual-trap optical tweezers trap fluorescent-labeled, microsphere-conjugated probes and measure the force induced on the probe as it is moved through a network of selectively-labeled entangled F-actin by using a nanoprecison piezoelectric stage to move the sample chamber relative to the traps. Fluorescence microscopy is used simultaneously to image the dynamics of the moving probe as well as the surrounding labeled molecules subject to the applied strain. Specific bioconjugation of the fluorescent polystyrene microspheres to the ends of labeled F-actin is achieved by carbodiimide attachment of gelsolin to microspheres and combining gelsolin-coated microspheres with fluorescent phalloidin labeled actin. This powerful single-molecule technique allows simultaneous measurement of intermolecular forces and dynamics and deformations of single molecules, providing the much needed link between stress and strain at the molecular level in complex F-actin networks.

1910-Pos Board B680

Dynamic of Stress Fibers in the Lamella of Spreading Fibroblasts

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A cell's contractile network and mechanosensing machinery involves actomyosin filaments connected to the ECM by focal adhesions. The initiation of these structures at the front of the lamella is therefore critical for various cell processes requiring motion, contraction, or signal transduction with the microenvironment. Though the properties of lamellipodial actin have been extensively studied, it is not fully known how the lamellipodium/lamella border is formed, and what happens at this interface. We track the formation, stabilization and dynamics of both stress fibers and focal adhesions using timelapse imaging of fibroblasts expressing actin and vinculin tagged with a fluorophore. Cells are seeded on specific adhesive micropatterns fabricated with a standard photolithography technique. We control the localization of adhesion sites and therefore the spreading geometry by alternating adhesive branches and non-adhesive gaps a few μm wide. We observe that above a given distance between adhesions, an actin bridge parallel to the leading edge and rich in myosinII is formed and reinforced. As the leading edge advances, nascent adhesions mature under the tension exerted by actin filaments. The latter stabilize into dorsal stress fibers, along which transverse arcs slide centripetally, eventually being either disassembled or fusing with dorsals to create a ventral stress fiber (connected to focal adhesions at both ends). After the initial spreading phase, we observe cytoskeletal remodeling. Ventral stress fibers are further aligned along one preferential axis to redistribute tension within the cytoskeleton and on adhesion sites.

Experimental data are used to build a simplified model of cell spreading, based on mechanical properties of stress fibers and focal adhesions. The simulations output will shed light on how these structures interact dynamically.

1911-Pos Board B681

A Two-Step Nuclear Mechanotransduction Model

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For cells to adapt to different tissues and changes in tissue mechanics, they must be able to respond to mechanical cues by changing gene expression patterns. These responses potentially involve major changes in nuclear organization and structure to reflect epigenetic changes in the nucleus. However, it is unclear how physical cues received at the plasma membrane integrate to the functional architecture of the cell nucleus. Recent evidence suggests that the nucleus responds to force via concurrent biochemical and mechanical pathways. Applying force to the plasma membrane of cells resulted in mechanical deformation of the nucleus and displacement of heterochromatin regions. Simultaneously, the actin cytoskeleton reorganized in response to force application, which further induced the translocation of transcription co-factor MKL from the cytoplasm to the nucleus. We present a minimal quantitative model incorporating active stresses and chemical kinetics to evaluate the reaction and timescales of nuclear reaction to force. Our work shows a minimal system of biochemical and mechanical effects working in tandem to recapitulate the observed effects of nuclear mechanosignaling.

1912-Pos Board B682

Nuclear Localization of mDia2 Formin and its Possible Regulation by Rho Family GTPases

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Formin family proteins are potent activators of actin filament nucleation and elongation. While numerous studies indicate the possible roles of actin inside the nucleus and the tight association of the nucleus with cytoplasmic actin cytoskeleton, the role of actin-associated proteins and, in particular, regulators of actin polymerization in the organization of nuclear and perinuclear actin remains obscure. Nuclear localization of some formins, such as formin-1 and mDia2 was detected in some previous studies. Particularly, it was recently shown that mDia2 has both nuclear localization and nuclear export signals, and is accumulated in the nucleus upon inhibition of nuclear export by leptomycin B (LMB) (Miki et al, J Biol Chem. 2009). We confirmed and extended these results; in particular, we have measured the kinetics of nuclear accumulation of some formins upon LMB treatment and have found that mDia2 accumulates in the nucleus very fast while mDia1 does not accumulate. Since mDia2 activity is regulated by the Rho family GTPases, we further studied the nuclear localization of RhoA, Rac1, and Cdc42 and found that significant amounts of the wild type Rho GTPases were localized to the nucleus, while